Journal of Shellfish Research, Vol. 24, No. 1, 301–308, 2005.

VARIATION IN FIBER, PROTEIN, AND LIPID CONTENT OF SHRIMP FEED—EFFECTS ON GUT PASSAGE TIMES MEASURED IN THE FIELD

J. J. BESERES,[1,*] A. L. LAWRENCE[2] AND R. J. FELLER[1,2]

[1]*Marine Science Program, Baruch Marine Field Laboratory, University of South Carolina, PO Box 1630, Georgetown, South Carolina 29442;* [2]*Shrimp Mariculture Project, Texas Agricultural Experiment Station, Texas A&M University, 1300 Port Street, Port Aransas, Texas 78373;*
[3]*Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208*

ABSTRACT The effect of varying levels of fiber, protein, and lipid feed component levels on gut passage time (GPT) and gut passage rate (GPR) of *Farfantepenaeus aztecus* (Pérez Farfante & Kensley 1997), *Litopenaeus setiferus* (Pérez Farfante & Kensley 1997), and *Litopenaeus vannamei* (Pérez Farfante & Kensley 1997), was examined in field feeding trials in a tidal creek and shrimp culture pond. Feeding trials were conducted in flow-through enclosures and feeds were thoroughly mixed with inert fluorescent latex beads to facilitate observation of the feed location within the guts of the shrimp. Rather than being able to continuously view feed passage through the shrimp guts (as is possible in the laboratory), we developed indirect methods that allowed us to obtain periodic "snapshots" of feed movement through shrimp guts at 10-min intervals, which were then used to calculate GPT and GPR. We expected to observe differences in GPTs because invertebrates are known to adjust their gut passage dynamics and GPTs should change as a function of food quality. Surprisingly, very large variations in feed component levels, whether fiber, protein, or lipid, did not cause any large differences is GPT within any of the three species. Mean GPTs ranged from 65.7–90.5 min in *F. aztecus* and *L. setiferus* and from 48.3–66.6 min in *L. vannamei*. GPRs were not constant, ranging from 5–16 mm/min when GPTs were short and from 0.1–2 mm/min for longer GPTs. Finding little change in GPTs with large changes in food quality was consistent with previous studies using other methods.

KEY WORDS: digestibility, *Farfantepenaeus aztecus*, gut passage time, *Litopenaeus setiferus*, *Litopenaeus vannamei*, shrimp feeding

INTRODUCTION

Feed and costs associated with feeding correspond to 0% to 50% of the variable production costs for aquaculture, depending on culture intensity, species, and feed management quality (Lawrence & Lee 1997). Knowledge of optimal feed formulation is necessary to attain maximum nutrient retention by the shrimp while reducing the amount of waste generated. Waste products (feces) and uneaten feed are the primary sources of inorganic and organic nutrients within aquaculture systems and discharged as effluent (Nunes & Parsons 1998). The associated degradation of water quality has been linked to reduced pond production through increased stress on the shrimp and susceptibility to disease (Tacon & Barg 1998). Soy-based feeds have been touted as potentially better, "cleaner" feeds as compared with fish-based feeds.

Because the length of time food remains in the gut can influence its digestibility (portion absorbed), we can examine the effects of food quality on more easily measured gut passage time (GPT). Previous research among different shrimp species, using different experimental methods, have provided conflicting results concerning how digestibility and GPT change with food quality (Lee 1971, Sedgwick 1979, Fair et al. 1980, Koshio et al. 1993, Stephen 2001, Glencross et al. 2002). Furthermore, much needed field measurements are scarce.

Changes in GPT maximize energy uptake, perhaps causing changes in assimilation efficiency (AE) and growth rate (Taghon 1981, Ahrens et al. 2001). We know that if ingestion rate = egestion rate, then assimilation efficiency will decrease as ingestion rate increases (Calow 1975, Calow 1977, Valiela 1995). Furthermore, if an organism's assimilation efficiency is approximately proportional to the digestibility of its food, then we can expect GPTs to change with changes in food quality.

*Corresponding author. E-mail address: beseres@biol.sc.edu

While the importance of nutritional requirements such as fiber, protein, and lipid in the diet of penaeids has been well established (Sick & Andrews 1973, Fair et al. 1980, Dall et al. 1990, Koshio et al. 1993, Lee & Lawrence 1997, Glencross et al. 2002), our knowledge of the basic mechanisms controlling gut passage within these species is still incomplete. Our null hypothesis assumed that shrimp GPTs would not change as a function of varying feed levels of protein, fiber, and lipid. Based on previous work, the alternative hypotheses were: (1) increasing GPT with increasing feed protein due to the high protein requirement of shrimp (Guillaume 1997; but see Lawrence & Lee 1997), some evidence for positive correlation with digestibility (Smith et al. 1985; Lee & Lawrence 1997); (2) increasing GPT with increasing feed fiber due to fiber's indigestibility and evidence of increased gut residence time with increased fiber (Shiau 1997); and (3) no change in GPT with increasing feed lipid content due to shrimp's relatively low lipid requirement (Teshima 1997).

We believe that information on how GPT is affected by changes in feed quality can provide aquaculture managers with data that may be used to improve existing but perhaps wasteful feeding regimes.

MATERIALS AND METHODS

This study was conducted at Oyster Landing Creek (33°21′2″ Lat., 79°11′27″ Long.), located near the Belle W. Baruch Marine Field Laboratory in Georgetown, South Carolina (*Farfantepenaeus aztecus* and *Litopenaeus setiferus*) and in two commercial shrimp mariculture ponds operated by Palmetto Aquaculture located in southeastern South Carolina (Beaufort, SC: 32°22′24″ Lat. 80°45′00″ Long. and Yemassee, SC: 32°37′24″ Lat. 80°52′30″ Long.) (*L. vannamei*). All three species have important commercial value, being harvested inshore and offshore in trawler fisheries. Additionally, *L. vannamei* and *L. setiferus* have been used for global aquaculture. Juvenile shrimp were collected from

the creek and ponds using 63 mm mesh seine and cast nets. Shrimp from a narrow size range were used in feeding trials (total length (TL, mm) = length from tip of rostrum to end of uropod).

Prior to conducting the feeding trials, *F. aztecus* and *L. setiferus* juveniles were held for 3–5 days in continuously-circulating seawater tanks with constant aeration and natural illumination. Seawater temperature (23°C to 29°C) and salinity (28–30 psu) fluctuated according to ambient weather and tidal conditions. During this period, shrimp were fed *ad libitum* twice daily with the base feed used in this experiment (Table 1). Feeding trials for *L. vannamei* juveniles were conducted immediately after their collection from the culture ponds, hence they had no prior exposure to the experimental feeds. Physical conditions in the ponds were similar to those in the tidal creek except for the absence of tidally-driven changes in water level.

Experimental Feeds

Shrimp GPTs were measured in feeding trials utilizing 13 different soy feeds varying in levels of fiber, protein, and lipid. The experimental feeds were grouped into three types (fiber, protein, or lipid), and each type contained 5 feed component levels (low, medium low, base, medium high, and high) with levels (proportions) of the manipulated component ranging as follows: Fiber feeds = 2.3% to 11.3% fiber by dry weight; Protein feeds = 20.1% to 45% protein by dry weight; and Lipid Feeds = 3.5% to 13.5% lipid by dry weight. All other components within these feeds remained essentially constant (Table 1). The midlevel (Base) feed was the same for all 3 feed types and contained component proportions of 30% protein, 7.5% lipid, and 5% fiber.

To facilitate observation of the feed location within the guts of the shrimp, feeds were thoroughly mixed (19 g feed: 0.03 g beads: 10 mL seawater) with inert fluorescent orange or pink latex beads (2–5μ diameter, specific gravity 1.40; Radiant Color, Richmond, CA). Labeled feeds were extruded through a pastry bag and shaped into 5-mm diameter pellets, similar in size to feeds used by Palmetto Aquaculture Co., (Columbia, SC). Extruded feed pellets were immediately sealed in plastic bags and frozen; they did not contain a binder.

TABLE 1.

Composition of 13 different soy-based feeds, varying independently in fiber (2.3 to 11.3% dry wt), protein (20.1% to 45% dry wt) and lipid (3.5% to 13.5% dry wt) levels. Feeds are listed in columns from left to right: base feed, fiber feeds, protein feeds, and lipid feeds. Total protein, fiber and lipid (% dry weight) are listed in rows for each feed, followed by total and digestible energy (kcal/kg feed), and ingredients (% dry weight) used in each feed.

Ingredients	Base Feed	Low Fiber	Med Low Fiber	Med High Fiber	High Fiber	Low Protein	Med Low Protein	Med High Protein	High Protein	Low Lipid	Med Low Lipid	Med High Lipid	High Lipid
Percent													
Total Protein	30.1	30.1	30.1	30.1	30.1	20.1	25.1	37.0	45.0	30.1	30.1	30.1	30.1
Total Fiber	5.3	2.3	3.8	7.8	11.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Total Lipid	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	3.5	5.5	10.0	13.5
kcal/kg													
Total Energy	4337	4210	4273	4441	4565	4460	4422	4838	4323	3962	4147	4570	4591
Digestible Energy	3194	3194	3194	3194	3194	3176	3192	3213	3160	2124	3069	3362	3593
Percent by dry weight													
Wheat starch	40.00	*	*	*	*	56.70	48.80	29.60	20.00	*	*	*	*
Soybean 91%	15.00	*	*	*	*	8.80	12.00	20.00	26.00	*	*	*	*
Casein vit free	7.20	*	*	*	*	*	*	*	*	*	*	*	*
Diatomaceous earth	6.00	9.00	7.50	3.50	0.00	0.00	2.60	9.00	10.00	10.00	8.00	3.50	0.00
Krill antarc chi	6.00	*	*	*	*	*	*	*	*	*	*	*	*
Wheat gluten	6.00	*	*	*	*	*	*	*	*	*	*	*	*
Oil soybean	4.00	*	*	*	*	*	*	*	*	*	*	*	*
Cellulose	3.00	*	*	*	*	*	*	*	*	*	*	*	*
PM min ain76	3.00	*	*	*	*	*	*	*	*	*	*	*	*
Cellulose, car.me	2.00	*	*	6.00	9.50	*	*	*	*	3.00	3.00	3.00	*
Fish sol cnd nrc	2.00	*	*	*	*	*	*	*	*	*	*	*	*
Phosphol 97%	1.50	*	*	*	*	*	*	*	*	*	*	*	*
PO4CaH monob	1.50	*	*	*	*	*	*	*	*	*	*	*	*
Oil fish men	0.80	*	*	*	*	*	*	*	*	*	*	*	*
NaCl reagent	0.50	*	*	*	*	*	*	*	*	*	*	*	*
PM min/vit beliz	0.40	*	*	*	*	*	*	*	*	*	*	*	*
Methionine	0.30	*	*	*	*	*	*	*	*	*	*	*	*
Arginine	0.26	*	*	*	*	*	*	*	*	*	*	*	*
Lysine	0.24	*	*	*	*	*	*	*	*	*	*	*	*
Cholesterol	0.20	*	*	*	*	*	*	*	*	*	*	*	*
Cit v stab 25%	0.10	*	*	*	*	*	*	*	*	*	*	*	*

Ingredients marked with an asterisk indicate no change from the ingredient level of the base feed.

Previous research utilizing similar-sized latex bead tracers in decapod, polychaete, and dipteran larvae, feeding experiments have shown that the latex beads did not move independently from the feed during gut transit and were present throughout the fecal strands (Wotton et al. 1998, Hoyt et al. 2000, Ahrens et al. 2001, Stephen 2001).

Experimental Enclosures

Gut passage time is defined as the elapsed time between first ingestion of labeled feed and its earliest or first defecation. Our feeding experiments were conducted in turbid waters, thus we could not directly observe shrimp. Rather than being able to continuously observe feed passage through the shrimp guts (as is possible in the laboratory), we developed indirect methods utilizing portable enclosures that allowed us to obtain periodic "snapshots" of feed movement through shrimp guts at 10-min intervals.

Enclosures were assembled as a 1.27-cm PVC frame (30 × 30 × 10 cm) covered with two layers of polyethylene mesh (0.47 cm inside, 0.64 cm outside). The bottoms contained only one layer of 0.64-cm mesh to allow shrimp contact with the sediment. Feeding trials were conducted using 2 replicate sets of 12 enclosures, each placed 15 m apart within the creek or pond. Within both replicate sets, 10 shrimp were placed into each enclosure along with the randomly selected feed of interest. Preliminary experiments confirmed that shrimp behavior and willingness to feed in the enclosures was not different from that exhibited by shrimp feeding in round aquaria in the laboratory.

Feeding Trials

Shrimp from the holding tanks were randomly selected and placed at densities of 10 per enclosure within 24 enclosures near the time of daytime low tide in Oyster Landing Creek (*Farfantepenaeus aztecus* and *Litopenaeus setiferus* trials) and within 18 enclosures in the Palmetto Aquaculture pond (*L. vannamei* trials). Preliminary observations revealed that *L. vannamei* gut passage of the labeled feeds was almost always complete after only 90 min, so the pond trials were truncated to 2 sets of just 9 enclosures. Pellets of the randomly selected feed were offered *ad libitum* to each of the enclosures, signifying time 0 for the feeding trial.

Every 10 min, one enclosure was removed from the downstream end of each replicate set (2 enclosures removed every 10 min). Shrimp in the Palmetto Aquaculture pond trials needed to be returned alive to the pond and thus were immediately examined under a dissecting microscope to record the "snapshot" of feed location in the gut; shrimp TL was also measured. Logistics of the Oyster Landing trials required that shrimp be held in the field before feed location could be recorded. These shrimp were placed in plastic bags submerged in crushed ice to halt feed passage through the gut. Shrimp observed with labeled feed at the anus when removed from the enclosure did not defecate when put on ice, as no fecal strands were recovered from the plastic bags. This demonstrated that egestion was indeed halted. Shrimp were held on ice until all enclosures and shrimp were removed from the creek (120 min), and then brought into the laboratory for examination.

The location of the leading edge of feed (closest to the anus) was recorded as follows: empty = 0; feed in proventriculus = 1; feed 1/4 way through intestine = 2; feed 1/2 way through intestine = 3; feed 3/4 way through intestine = 4; feed at anus = 5. The locations of nonlabeled food items present in the gut were also noted.

All feeding trials were conducted during daylight hours. However, to test whether diurnal changes affect GPT, the low protein feed was also offered to *L. vannamei* during the night.

Indirect Determination of Gut Passage Time

Observations of feed located at the anus (score = 5) at 10-min intervals were used to calculate mean GPT for each feed using the equation:

$$\text{Mean GPT (min)} = \sum [(T-5)n] / N,$$

where T is the time (min) when shrimp were removed from the creek for observation of feed location (e.g., 50 min after feed was offered), n is the number of shrimp for that time period that had feed located at the anus (score = 5), and N is the total number of shrimp that had feed located at the anus summed across all time periods. We used a value of T − 5 (= midpoint of current time interval) assuming that, if feed is located at the anus, defecation occurred sometime after the end of the previous time interval but before the end of the current time interval, T.

We examined the relationship between GPT and GPR to determine whether feed passed through the gut at different rates over different lengths of GPT. Gut passage rates (GPR, mm/min) were calculated as:

$$\text{GPR} = \text{GL/GPT},$$

where gut length (GL, mm) was calculated as:

$$\text{GL} = 0.75\ \text{TL (Clark 2000)}$$

Statistical Analysis

StatView 5.0.1 for Windows (SAS Institute) was used for all statistical analyses. All analyses were conducted at the 95% confidence level. Although the shrimp used were from a narrow size range, we included TL as a factor in the ANCOVA models to check for any large differences in GPT that might have been due to small differences in TL. We first used an ANCOVA procedure to compare GPTs for a feeding trial between replicate sets of enclosures using the model: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where μ = grand mean GPT, α_i = main effect of replicate set, β_j = main effect of TL, $(\alpha\beta)_{ij}$ = interaction of replicate × TL, and ε_{ijk} = error term. Finding no such differences, data were pooled from both replicate sets of enclosures for subsequent analyses.

For each of the 3 feed types offered to each species, we next used an ANCOVA model that included both component level (5 levels) and shrimp size (TL, mm) to test for their effects on differences in GPT using the model: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk,}$, where μ = grand mean GPT, α_i = main effect of feed component level, β_j = main effect of TL, $(\alpha\beta)_{ij}$ = interaction of feed type × TL, and ε_{ijk} = random error. If the results of this full ANCOVA showed no significant effect of TL and no significant interaction between feed level and TL, then TL was dropped from the model. This reduced model ANCOVA was then used to test for differences in GPT as a function of differences in feed component level. The Fisher protected least significant difference (PLSD) pairwise comparison test was used to identify which GPTs were significantly different from one another within a shrimp species.

The Kolmogorov–Smirnov 2-sample test was used to test for pairwise differences between the cumulative distributions of shrimp with feed at the anus over each trial period. The test is sensitive to differences in shape, location, and skewness of the distributions (Sokal & Rohlf 1995) and determines whether the maximum difference between cumulative distributions of two samples is significant.

RESULTS

A total of 35 feeding trials were conducted. Thirteen feeds were used in feeding trials with *Farfantepenaeus aztecus* and *Litopenaeus vannamei*; due to low shrimp abundance in the creeks during the summer of 2001 only 8 feeds were used in feeding trials with *L. setiferus*. Shrimp recovery and feeding success were high for all 3 species. Recovery was 92% (2880/3120) for *F. aztecus*, 94% of those recovered contained labeled feed in the gut (only 6% contained no labeled feed), and 39% of these had feed at the anus (score = 5) at some time during the feeding trial. Recovery was 87% (1670/1920) for *L. setiferus*, 92% of those recovered contained labeled feed in the gut (8% contained no labeled feed), and 41% of these had score = 5. Recovery was 92% (2322/2520) for *L. vannamei*, 86% of those recovered contained labeled feed in the gut (14% contained no labeled feed), and 35% of these had score = 5. Mean gut passage times were calculated for all shrimp collected from the enclosures that had feed located at the anus (score = 5). For all species and all feeding trials, there were no significant differences in GPTs that could be ascribed to any differences in the size (TL) of shrimp.

The fluorescent latex bead label remained integrated with the feeds (did not separate) while passing through the gut. When fecal strands were recovered, they likewise contained an undifferentiated mixture of beads and egested feed. Once food moved out from the proventriculus, it moved as a single "pulse" without breaks or empty spaces in the intestine.

Temperatures of the creek and pond water during the feeding trials were consistent across all trials and reflected natural warming throughout the summer. Temperatures (mean ± SD) for *F. aztecus* were 28.1 ± 2.6°C, for *L. setiferus* 29.4 ± 2.1°C, and for *L. vannamei* 29.7 ± 1.4°C. Salinities were also similar between feeding trials, with values (mean ± SD) of 30.5 ± 0.3 psu for *F. aztecus*, 25.9 ± 2.0 psu for *L. setiferus*, and 29.7 ± 1.4 psu for *L. vannamei*.

Effect of Feed Component Levels on Gut Passage Time

We expected to see some differences in GPTs for two main reasons: first, invertebrates are known to vary their GPTs, and second, GPT is linked to food quality. Comparing GPTs across feed component levels in any one of the species, we did not expect to find such low variation in the time it took for labeled feeds to move through the gut. Surprisingly, very large variations in feed component levels, whether fiber, protein or lipid, did not cause any large differences in GPT within any of the three species (Table 2).

For *Farfantepenaeus aztecus*, none of the GPTs measured using all 13 of the feeds were significantly different from one another within feed type.

For *L. setiferus*, GPTs were similar between the two lipid feeds tested, and between 4 of the 5 protein feeds; only the medium low protein feed resulted in a significantly shorter GPT ($P = 0.0187$). GPTs for *L. setiferus* on the 3 fiber feeds tested were similar except for significantly longer GPT for the high fiber feed than for the low fiber feed ($P = 0.0009$).

For *L. vannamei*, GPTs were similar for all 5 of the lipid feeds and for 4 of the 5 fiber feeds; only the medium high fiber feed resulted in a significantly shorter GPT ($P < 0.0001$). Although a significant statistical interaction was detected between feed component level and TL for the protein feeds ($P = 0.0269$), using the Fisher PLSD posthoc tests, *L. vannamei* GPTs were similar between 3 of the 5 protein feeds. GPTs from ingestion of the low protein feed were significantly shorter than for the other protein feeds ($P < 0.0001$), and GPTs from the medium high protein feed were significantly shorter ($P = 0.0149$) than the lower protein feeds (but not from the high protein feed).

There was also no significant diurnal difference in GPT for *L. vannamei* using the low protein feed on two different dates. The daytime trial was conducted June 23 (14:30 local time), and the nighttime trial was conducted August 18, 2004 (23:00 local time).

TABLE 2.

Mean gut passage times and mean total length for field feeding trials with juvenile *F. aztecus, L. setiferus* and *L. vannamei* on feeds varying in levels of fiber, protein or lipid. Post-hoc pairwise comparisons were conducted with Fisher's PLSD (Protected Least Significant Difference) test; within feed types (fiber, protein, lipid) for each species.

	F. aztecus				*L. setiferus*				*L. vannamei*			
Feeds	GPT (min)	± SD	TL (mm)	± SD	GPT (min)	± SD	TL (mm)	± SD	GPT (min)	± SD	TL (mm)	± SD
Fiber Feeds												
Low	73.6[a]	5.4	53.3[a]	9.9	74.2[b]	5.3	62.3[b]	11.6	66.6[d]	7.9	89.7[d]	7.5
MedLow	78.3[a]	7.4	56.2[a]	12.7	no data				62.3[d]	6.4	96.8[d]	6.2
Base	75.4[a]	5.6	47.9[a]	11.6	81.0[b,c]	6.4	63.7[b]	8.3	66.6[d]	9.1	95.2[d]	10.3
MedHigh	72.5[a]	5.2	50.9[a]	9.2	no data				48.3[e]	3.9	87.0[d]	7.8
High	75.4[a]	5.7	54.0[a]	9.0	90.5[c]	10.5	91.5[b]	9.7	59.5[d]	5.9	90.7[d]	8.6
Protein Feeds												
Low	70.8[a]	5.1	49.4[a]	8.7	76.4[b]	5.5	65.6[b]	10.4	48.2[d]	4.1	101.2[d]	8.2
MedLow	76.3[a]	5.8	57.0[a]	8.7	65.7[c]	4.9	62.8[b]	7.4	64.1[e]	7.4	90.2[d]	9.3
Base	75.4[a]	5.6	47.9[a]	11.6	81.0[b]	6.4	63.7[b]	8.3	66.6[e]	9.1	95.2[d]	10.3
MedHigh	78.9[a]	6.2	54.5[a]	10.0	77.2[b]	6.0	62.7[b]	9.4	55.6[f]	5.0	90.8[d]	8.0
High	78.4[a]	6.4	56.2[a]	11.3	79.2[b]	6.6	64.8[b]	11.2	59.1[e,f]	6.2	103.8[d]	8.0
Lipid Feeds												
Low	76.6[a]	5.4	54.7[a]	10.6	76.4[b]	5.4	83.0[b]	12.4	63.7[d]	7.4	91.2[d]	8.3
MedLow	68.9[a]	5.1	53.0[a]	7.7	no data				66.5[d]	8.6	101.0[d]	6.4
Base	75.4[a]	5.6	47.9[a]	11.6	81.0[b]	6.4	63.7[b]	8.3	66.6[d]	9.1	95.2[d]	10.3
MedHigh	80.8[a]	8.4	52.7[a]	9.2	no data				55.0[e]	6.7	99.8[d]	10.6
High	72.8[a]	5.1	48.5[a]	8.7	no data				61.1[d,e]	6.3	90.6[d]	8.2

GPTs and TLs with different letters are significantly different from one another at $P < 0.05$.

GPTs from the daytime trial (48.2 ± 4.1min) (mean ± SD) were remarkably similar to GPTs from the nighttime trial (52.5 ± 5.2 min).

L. vannamei GPTs were consistently shorter than those of *F. aztecus* or *L. setiferus* (Fig. 1); unpredictably, GPTs from the latter 2 species were within a similar range. Within all 3 species, GPTs did not show a consistent trend of increasing or decreasing with increasing feed component levels.

The cumulative frequency distributions of the percentages of shrimp sampled through time, whose intestines contained labeled feed at the anus (score = 5), illustrate high similarities (Fig. 2). Regardless of species, it took about 30 min before a significant number of animals achieved initial gut transit of labeled feed. The similarities in the slopes of the cumulative percentage curves indicate that shrimp did not process any of the different feeds more rapidly than others. The Kolmogorov–Smirnov 2-sample test results confirm this visual impression, finding no statistically significant differences across any of the three different feed types within each species ($P > 0.05$).

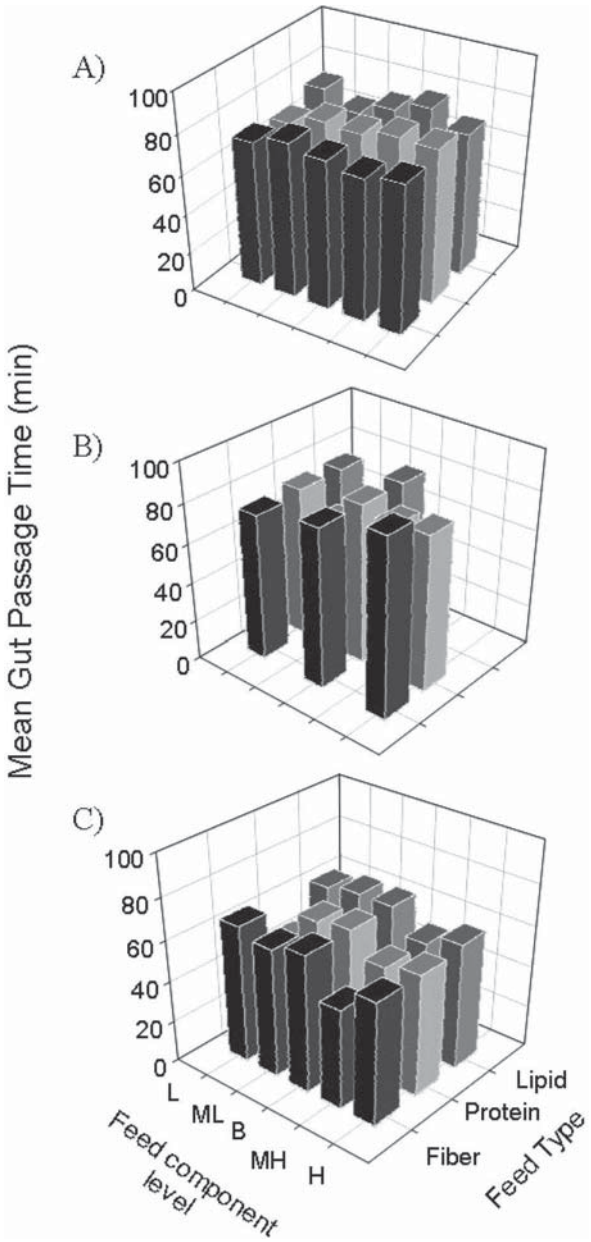

Figure 1. Mean GPTs for (A) *F. aztecus*, (B) *L. setiferus*, and (C) *L. vannamei* on fiber, protein, and lipid feeds ranging from Low to High levels of the component of interest. L = Low, ML = Medium Low, B = Base, MH = Medium High, H = High.

Effect of Feed Component Levels on Gut Passage Rates

The relationships between GPR and GPT were fit using inverse first-order regressions with the equation:

$$y = y_o + a / x$$

Where y = GPR, y_o = asymptote, a = y-intercept, x = GPT. For *Farfantepenaeus aztecus*, $y_o = 0.0066$ and a = 39.17; for *Litopenaeus setiferus*, $y_o = -0.0246$ and a = 53.17; for *L. vannamei*, $y_o = 0.0188$ and a = 69.91 (Fig. 3).

The relationships showed remarkable similarity between species, although GPRs were consistently higher for *L. vannamei* than for the other two species. For all species, GPR exhibited greater variability and sensitivity to small changes in GPT at short GPTs, while maintaining small variability and approaching an asymptote at longer GPTs. There was no significant correlation between GPR and TL for any of the 13 feeds tested ($r^2 = 0.018$ *F. aztecus*, = 0.024 *L. setiferus*, = 0.005 *L. vannamei*).

DISCUSSION

Effect of Feed Component Levels on Gut Passage Time

Although several studies have examined relationships between various feeding dynamics and food quality, the conclusions are often conflicting because different species and different methodologies have been applied. The trends we found of only minor changes in GPTs with changing feed component levels were unexpected but consistent with the findings of others. There are few studies of GPT for direct comparison with our results, and we acknowledge that the supposed relationships (if any) between GPT and growth or digestibility or even assimilation efficiency need to be further established. Clearly, much more research in this arena is needed for penaeids.

Knowledge of the relationship between GPT and changes in feed quality is important for identifying favorable feed formulations and developing efficient feeding regimes for cultured shrimp. Our study unpredictably demonstrated that large variations in feed fiber, protein, or lipid levels did not cause large differences in GPT for *Farfantepenaeus aztecus*, *Litopenaeus setiferus* or *L. vannamei*. Regardless of feed quality, there was minimal variation in the time it took for the labeled feeds to move through the gut. The values we obtained for GPT agree with Lee and Lawrence (1997), who reported that the penaeid proventriculus can be filled after 1–10 min of eating, and that foregut clearance can be 75% complete within 1 h. Similar results were also found for *Penaeus esculentus*, *P. monodon*, *P. stylirostris*, *P. californiensis*, and *P. vannamei* (Dall et al. 1990).

Penaeids in the wild obtain most of their energy requirements from dietary protein (Dall et al. 1990), and commercial feeds are regularly formulated with elevated protein percentages to promote faster shrimp growth. We had expected an increase in GPT with increasing feed protein levels, assuming that feeds of higher digestibility require longer GPT. Our results demonstrated that GPT was not significantly affected by changes in feed protein level for

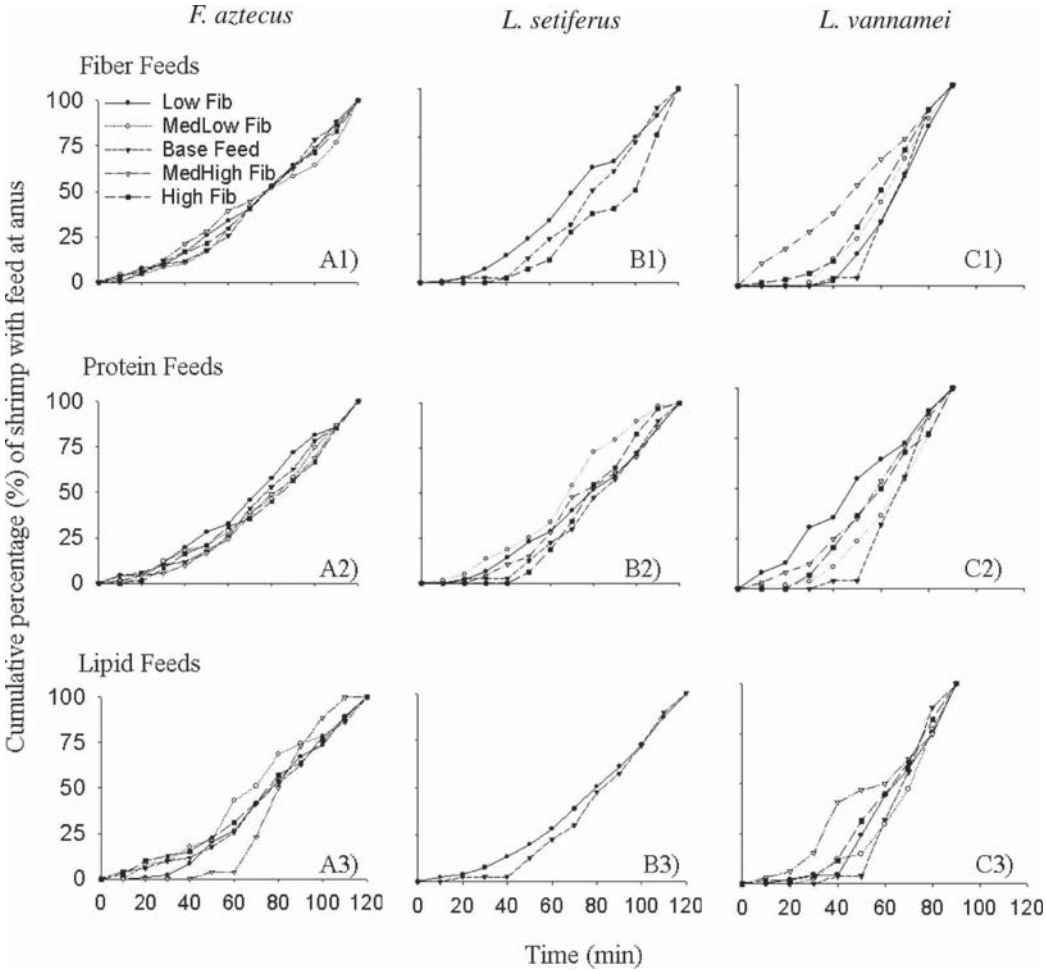

Figure 2. Cumulative percentage of shrimp with feed at anus (score = 5) over each 10-min time interval for *F. aztecus* (A1 to A3), *L. setiferus* (B1 to B3), and *L. vannamei* (C1 to C3) fed 5 the same fiber, protein, and lipid feeds ranging from L to H levels over 120-min (*F. aztecus* and *L. setiferus*) or 90-min (*L. vannamei*) field feeding trials.

any of the three species. This finding is consistent with Lee (1971), who found that *Penaeus monodon* had similar digestibilities for dietary protein levels ranging from 33% to 62%. Sedgwick (1979) found no change in *P. merguiensis* growth when feed protein content was reduced from 51% to 34%. Similarly, Koshio et al. (1993) reported that *P. japonicus* had similar weight gain, specific growth rate, and feed conversion efficiency over a similar range of protein levels (21% and 31.4%) as used in the present study. However, within that same study, Koshio et al. (1993) found that specific growth rates, weight gain and feed conversion efficiencies were higher for *P. japonicus* feeding on 41.6, 50.3, and 60.7% protein feeds as compared with the 21% and 31.4% feeds. Interestingly, Deshimaru and Yone (1978) found those diets containing either extremely low (2% and 11.2%) or extremely high protein (66.2%) remarkably depressed the growth of *P. japonicus*. For the range of protein levels used in our study, most results indicate that neither GPT nor digestibility was significantly affected by changes in feed protein level.

Although penaeids obtain less of their energy requirement from carbohydrates than from proteins (Dall et al. 1990), we had expected to see an increase in GPT as feed fiber levels increased due to its high indigestibility (Fair et al. 1980, Lee & Lawrence 1997, Shiau 1997). Increases in dietary fiber have been demonstrated to increase gut residence time (Shiau 1997) and decrease net assimilation (Fair et al. 1980). Thus, with increased fiber, we expected that the shrimp would need to slow down gut passage to allow for digestion of essential nutrients within the less digestible material before its defecation. Instead, we found that GPT was not significantly affected by large changes in feed fiber levels for any of the three species. This supports Fair et al. (1980), who found no significant increase in growth for juvenile *Macrobrachium rosenbergii* (0.08 ± 0.002 g) on feeds with 0, 5, 15, and 30% dietary fiber concentrations. They concluded that dietary fiber could either increase or decrease the rate of food passage through the gut, due either to its nonassimilable nature, or depending on which cellulases were present in the prawn digestive system.

Because penaeids have a relatively low requirement for lipid (Teshima 1997), and lipid is highly digestible, we did not expect to see a significant change in GPT due to variation in feed lipid levels. Our results supported this hypothesis; we did not see a significant effect of feed lipid level on GPT for any of the three species. This also supports Glencross et al. (2002) who reported *Penaeus monodon* had similar digestibilities for dietary lipid levels of 4.5, 7.5, and 10.5%. However, that same study demonstrated a relative decrease in lipid digestibility by *P. monodon* for high lipid feeds (13.5%). Our results conflict with kinetic digestion models

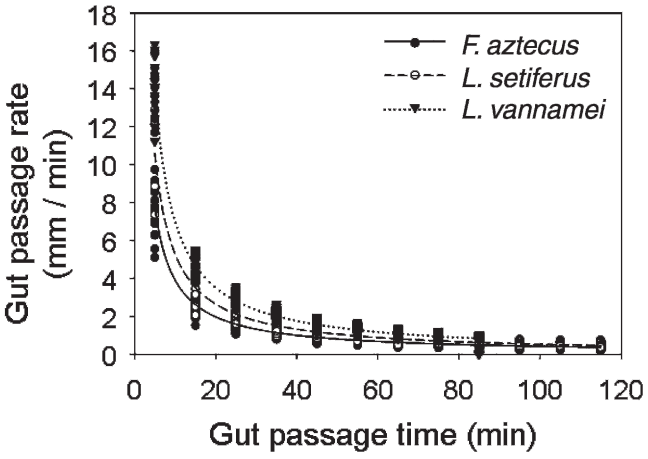

Figure 3. Best fit inverse first order regressions of gut passage rate (GPR) as a function of gut passage time (GPT) for *F. aztecus*, *L. setiferus* and *L. vannamei* in field feeding trials using all fiber, lipid, and protein feed types tested.

which suggest that increased levels of dietary fats should slow gastric emptying in invertebrates (Jumars 2000).

In our study, GPTs were calculated from intermittent "snapshots" of feed movement through the gut. These values were obtained from *Farfantepenaeus aztecus* and *Litopenaeus setiferus* that were previously collected in the tidal creek and held for a short time in the laboratory while being fed exclusively the experimental base feed. *L. vannamei* were used immediately after collection from the culture pond where high-protein commercial fish feed was provided approximately hourly with automatic broadcast feeders. Casual observations indicated that shrimp from both the creek and pond locations had natural food items in the gut at the start of the feeding trial and thus were not starving beforehand. This is important to consider when making inferences to other conditions, because starved shrimp tend to ingest food more rapidly than shrimp that have had feed continuously available (Dall et al. 1990).

The percentage of nonfeeding shrimp in our trials was very low, thus palatability of the feed to the target species was high. Further, even though *L. vannamei* did not have previous access to the experimental feeds, the percentage of these shrimp that fed on our experimental feeds was also very high. The soy-based feeds were readily accepted by all 3 species.

Estimations of GPT are complex in natural creek and pond locations because changes in water quality and availability of natural foods can affect feeding behaviors and gut passage of experimental feeds. We agree with Lawrence and Lee (1997) that although laboratory studies of feeding dynamics have enhanced our understanding of feed formulations, development and utilization of more natural experimental systems is needed. Measuring shrimp GPTs in their natural environment allows for comparisons with laboratory-measured GPTs, and provides data for evaluating the reliability of extrapolating laboratory measurements to natural field populations. As a continuation of this project, we compared the present study's GPT results with GPT results found in laboratory feeding experiments. We found close agreement between the GPT results, obtained with "snapshot" indirect measurements and direct observations of feed movement through shrimp in the laboratory (Beseres et al. submitted).

Shrimp in this study were able to access natural benthic foods through the cage bottom. Observations in the laboratory confirmed that indeed some shrimp had nonfeed items (nonlabeled natural food) present in the gut. However, once labeled feed was consumed and observed in the gut, the proportion of nonlabeled feed in the gut was always much less than that of the labeled feed, suggesting perhaps that the nonfeed items were less desirable. Gut passage time did not change even when natural foods of vastly different quality were mixed in with the labeled feeds. Shrimp raised in culture ponds for commercial harvest will always have access to detritus and benthic food sources, and thus investigations considering the relationship between GPT and feed quality without consideration of natural pond and creek food resources may be incomplete.

We anticipated finding differences in GPT between species. In particular, in preliminary observations in the laboratory, we noted that *L. vannamei* was a very aggressive feeder, seizing and defending feed pellets, feeding voraciously, and even attacking and eating other shrimp when feed was abundant. Results from the present study demonstrated that *L. vannamei* GPTs, regardless of feed quality, were on average about 10–20 min shorter than GPTs for the other two species. These results may be due, at least in part, to the aggressive feeding behaviors used by *L. vannamei*.

Effect of Feed Component Levels On Gut Passage Rate

It is not clear how penaeid GPR is affected by changes in food quality. Counter intuitively, GPRs were not correlated with shrimp size for any of the three species; smaller shrimp were not passing feed through their gut any faster than larger shrimp. This finding agrees with Nunes and Parsons (2000), who found that foregut clearance rates were similar regardless of shrimp size (mean 3.7–13.8 g) for *Penaeus subtilis*. Thus, shrimp within the size range examined in the present study 1–2 g (*Farfantepenaeus aztecus*); 2–5 g (*Litopenaeus setiferus*); 7–11 g (*L. vannamei*) may have been able to control their rate of food passage relative to parameters that were not measured as part of this study.

Knowledge of GPT dynamics allows pond managers to more closely match delivery of their daily rations to shrimp feeding behavior (Feller 1991, 1998). Our study did not measure the time needed for complete evacuation of a feed meal from the gut. However, based on our values for GPT (consistently around 80 min) for *F. aztecus* and *L. setiferus*, we believe it is reasonable to assume that gut evacuation is complete in about double that time (about 3 h) after feeding on these experimental feeds. For pond managers, it may be more efficient to deliver feeds every 3 h to allow time for complete gut passage of the feed as a way to reduce or eliminate excess uneaten feed waste. A slightly shorter interval (2 h) may be reasonable for *L. vannamei* to accommodate their shorter GPTs (~60 min) and to discourage cannibalistic behavior.

Omnivorous penaeids are clearly adapted to feeding on a variety of foods that range widely in their protein and energy content (e.g., from detritus to polychaetes), the latter having as much as 80% protein by dry weight (Allan & Smith 1998). Therefore, one might expect high variability in GPR for penaeids. More research is warranted to determine the effects of changes in dietary protein on penaeid GPT, GPR, and growth.

The results from our study are especially relevant in the context of choosing appropriate feed component levels for pond culture. Pond managers often use elevated protein levels to promote faster shrimp growth within their ponds, but the waste products from underutilization and leaching of high protein feeds can degrade water quality. Koshio et al. (1993) found that cumulative ammonia

excretion of *Penaeus japonicus* was positively correlated with dietary protein content. Further, optimal protein efficiency (ratio of protein retention to protein intake) has been observed in shrimp consuming feeds with protein levels below "optimal" growth levels (Millikin et al. 1980, Shiau et al. 1991). Based on these results and those from the present study, we believe it reasonable to suggest that reduced feed protein levels could be incorporated in shrimp pond culture, especially if they result in acceptable growth rates.

ACKNOWLEDGMENTS

The authors thank Drs. D. M. Allen, J. M. Dean, and S. E. Stancyk for their advice, and Mr. D. Dunkelberger of Palmetto Aquaculture and Mr. and Mrs. W. Campbell for use of the shrimp ponds. The authors also thank the field assistants S. Hagins, L. Maliszewski, T. Fitzhenry, M. Jaskowski, F. Helies, J. Housand, K. Palmigiano, B. Gaskins, J. Pollack, K. Fedorka, K. Bretsch, and J. Stephen. This study partially fulfilled requirements for the M.Sc. degree in the Marine Science Program, University of South Carolina, for J. J. Beseres. This research was sponsored by the South Carolina Sea Grant Consortium pursuant to National Oceanic and Atmospheric Administration Award No. R/A-32. The authors also acknowledge funding from the Slocum-Lunz Foundation in Charleston, SC, and from the South Carolina Wildlife Federation (to JJB) for this project.

LITERATURE CITED

Ahrens, M. J., J. Hertz, E. M. Lamoureux, G. R. Lopez, A. E. McElroy & B. J. Brownawell. 2001. The effect of body size on digestive chemistry and absorption efficiencies of food and sediment-bound organic contaminants in *Nereis succinea* (Polychaeta). *J. Exp. Mar. Biol. Ecol.* 263(2): 185–209.

Allan, G. L. & D. M. Smith. 1998. Recent nutrition research with Australian penaeids. *Rev. Fish. Sci.* 6(1-2):113–127.

Calow, P. 1975. Defecation strategies of two freshwater gastropods, *Ancylus fluviatus* Müll. and *Planorbis contortus* Linn. (Pulmonata) with a comparison of field and laboratory estimates of food absorption rate. *Oecologia* 20:51–63.

Calow, P. 1977. Ecology, evolution and energetics: a study in metabolic adaptation. *Adv. Ecol. Res.* 10:1–62.

Clark, O. M. 2000. Feeding dynamics of postlarval penaeid shrimp: gut passage time, prey composition and daily ration. M.Sc. Thesis, University of South Carolina. 128 pp.

Dall, W., B. J. Hill, P. C. Rothlisberg & D. J. Sharples. 1990. The biology of the penaeidae. Adv. Mar. Biol. Vol. 27.

Deshimaru, O. & Y. Yone. 1978. Optimum level of dietary protein for prawn. *Bull. Jpn. Soc. Sci. Fish.* 44(12):1395–1397.

Fair, P. H., A. R. Fortner, M. R. Millikin & L. V. Sick. 1980. Effects of dietary fiber on growth, assimilation and cellulase activity of the prawn (*Macrobrachium rosenbergii*). *Proc. World Maric. Soc.* 11:369–381.

Feller, R. J. 1991. Dietary analysis of penaeid shrimp: the immunoassay approach. In: P. Deloach, W. J. Doherty & M. A. Davidson, editors. Frontiers of shrimp research. Amsterdam: Elsevier Science Publishers. pp. 141–154.

Feller, R. J. 1998. Estimating gut passage times: relevance for feeding regimes in cultured shrimp. In: D. E. Jory, editor. Proceedings, First Latin American Shrimp Farming Congress, 6–10 October, 1988. Panama. pp. 1–7.

Glencross, B. D., D. M. Smith, M. R. Thomas & K. C. Williams. 2002. The effects of dietary lipid amount and fatty-acid composition on the digestibility of lipids by the prawn, *Penaeus monodon. Aquaculture* 205(1–2):157–169.

Guillaume, J. 1997. Protein and amino acids. In: L. R. D'Abramo, D. E. Conklin & D. M. Akiyama, editors. Crustacean nutrition. Baton Rouge, LA: World Aquaculture Society. pp. 26–50.

Hoyt, M., J. W. Fleeger, R. Siebeling & R. J. Feller. 2000. Serological estimation of prey-protein gut-residence time and quantification of meal size for grass shrimp consuming meiofaunal copepods. *J. Exp. Mar. Biol. Ecol.* 248(1):105–119.

Jumars, P. A. 2000. Animal guts as ideal chemical reactors: maximizing absorption rates. *Am. Nat.* 155(4):527–543.

Koshio, S., S. Teshima, A. Kanazawa & T. Watase. 1993. The effect of dietary-protein content on growth, digestion efficiency and nitrogen-excretion of juvenile Kuruma prawns, *Penaeus japonicus. Aquaculture* 113(1–2):101–114.

Lawrence, A. L. & P. G. Lee. 1997. Research in the Americas. In: L. R. D'Abramo, D. E. Conklin & D. M. Akiyama, editors. Crustacean nutrition. Baton Rouge, LA: World Aquaculture Society. pp. 566–587.

Lee, D. L. 1971. Studies on the protein utilization related to growth of *Penaeus monodon* Fabricius. *Aquaculture* 1:1–13.

Lee, P. G. & A. L. Lawrence. 1997. Digestibility. In: L. R. D'Abramo, D. E. Conklin & D. M. Akiyama, editors. Crustacean nutrition. Baton Rouge, LA: World Aquaculture Society, pp. 194–260.

Millikin, M. R., A. R. Fortner, P. H. Fair & L. V. Sick. 1980. Influence of dietary protein concentration on growth, feed conversion and general metabolism of juvenile prawn (*Macrobrachium ohione*). *Proc. World Maric. Soc.* 8:699–704.

Nunes, A. J. P. & G. J. Parsons. 1998. Dynamics of tropical coastal aquaculture systems and the consequences to waste production. *World Aquac.* 29(2):27–37.

Nunes, A. J. P. & G. J. Parsons. 2000. Size-related feeding and gastric evacuation measurements for the Southern brown shrimp *Penaeus subtilis. Aquaculture* 187(1–2):133–151.

Pérez Farfante, I. & B. Kensley. 1997. Penaeoid and sergestoid shrimps and prawns of the world; keys and diagnoses for the families and genera. *Memoires du Museum National d'Histoire Naturelle, Tome* 175:233 pp.

Sedgwick, R. W. 1979. Effect of ration size and feeding frequency on the growth and food conversion of juvenile *Penaeus merguiensis* de Man. *Aquaculture* 16:279–298.

Shiau, S. 1997. Carbohydrates and fiber. In: L. R. D'Abramo, D. E. Conklin & D. M. Akiyama, editors. Crustacean nutrition. Baton Rouge, LA: World Aquaculture Society, pp. 108–122.

Shiau, S. Y., C. C. Kwok & B. S. Chow. 1991. Optimal protein dietary level of *Penaeus monodon* reared in seawater and brackish water. *Nippon Suisan Gakkaishi* 57:711–716.

Sick, L. V. & J. W. Andrews. 1973. The effect of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum. Proc. World Maric. Soc.* 4:263–276.

Smith, L. L., P. G. Lee, A. L. Lawrence & K. Strawn. 1985. Growth and digestibility by 3 sizes of *Penaeus vannamei* Boone—Effects of dietary-protein level and protein-source. *Aquaculture* 46(2):85–96.

Sokal, R. R. & F. J. Rohlf. 1995. Biometry. 3rd ed. New York: W. H. Freeman and Company.

Stephen, J. 2001. Gut passage time, nitrogen assimilation efficiency, and growth in the white shrimp, *Litopenaeus setiferus*. M. Sc. Thesis, University of South Carolina. 109 pp.

Tacon, A. G. J. & U. C. Barg. 1998. Major challenges to feed development for marine and diadromous finfish and crustacean species. In: S. S. De Silva, editor. Tropical mariculture. New York: Academic Press. pp. 171–207.

Taghon, G. L. 1981. Beyond selection: optimal ingestion rate as a function of food value. *Am. Nat.* 118(2):202–214.

Teshima, S. 1997. Phospholipids and sterols. In: L. R. D'Abramo, D. E. Conklin & D. M. Akiyama, editors. Crustacean nutrition. Baton Rouge, LA: World Aquaculture Society. pp. 85–107.

Valiela, I. 1995. Marine ecological processes. New York: Springer-Verlag.

Wotton, R. S., B. Malmquist, T. Muotka & K. Larsson. 1998. Fecal pellets from a close aggregation of suspension-feeders in a stream: An example of ecosystem engineering. *Limnol. Oceanogr.* 43:719–725.